(54) Title: A METHOD OF DIFFERENTIATING PARTICLES BASED UPON A DYNAMICALLY CHANGING THRESHOLD
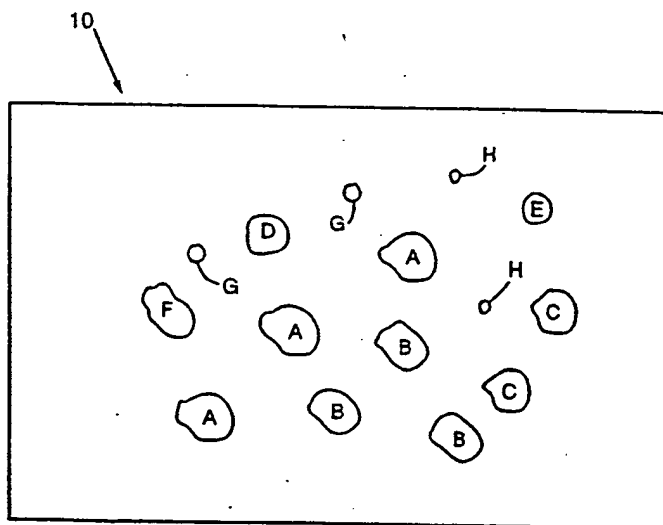
(57) Abstract

A first paramater is measured for each of the plurality of different particles. One of the type of particles (A) is identified based upon the first parameter measured. Once the one type of particle is identified, a second parameter, different from the first parameter, is then measured for the one type of particle. The other types of particles (B) in the field of view can be identified based upon the second parameter associated with the one type of particle which has been identified. In one example, color is first used to differentiate various different particles in the field of view and to identify one particular type of particle. Thereafter, a morphological parameter, such as size, associated with the one type of particle identified is measured. The measured morphological character associated with the one type of particle identified is used as a threshold to differentiate other particles in the field of view based upon the second morphological parameter. Similarly, a morphological characteristic can first be used to differentiate one type of particle. A second spectral parameter, such as color, can be measured for the one type of identified particle and can be used to differentiate other particles.

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A METHOD OF DIFFERENTIATING PARTICLES BASED UPON A
DYNAMICALLY CHANGING THRESHOLD

Technical Field

The present invention relates to a method of
differentiating particles from a plurality of
particles, based upon a threshold which is
dynamically changing. More particularly, the present
invention relates to a method of differentiating
particles wherein the threshold to differentiate the
particles is determined by a measurement using yet
another parameter.

Background Of The Invention

Methods to identify different particles in a
field of view are well-known in the art. See, for
example, U.S. Patent No. 4,175,860. Typically, the
particles that are under examination are biological
particles, and it is desired to automatically
identify the different particles under examination.

One of the problems faced by the prior art is
that various parameters (such as color) associated
with each particle can vary from one sample to
another sample. This variation can be caused, for
example, by different stains used in the preparation
of different samples or, by the age of the samples.
Thus, the same type of particle appearing in
different images of view may exhibit different
parameters.

One solution that has been offered by the prior
art is to add a normalizing substance which has a
predetermined, _a priori_ detection level. Thus, for
example, calibrator particles, as disclosed in U.S.
Patent No. 4,338,024 can be added to a sample. Based

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upon the a priori knowledge of the color of the
beads, if the color of the particle under examination
exceeds that threshold, then that particle is
identified as of a particular type. Clearly, such a
5   normalizing substance is inadequate because the
parameter of the particle can vary from sample to
sample (due to the factors of different stain and
aging as discussed above), whereas the parameter of.
the normalizing substance does not.

10   In another example of the prior art solution,
the DNA of all the cells are measured. A normalizing
value which is manually selected for the particular
sample lot for that experiment is selected and that
threshold is then used to identify the particles
15   whose DNA exceeds that threshold or is below the
threshold. With this approach, while the threshold
can vary, it requires manual interpretation and
intervention to determine the particular threshold
adequate for each of these experiments.

20   Summary Of The Invention
        In the present invention, a method of
identifying a plurality of different particles in a
field of view is disclosed. The method comprises the
steps of measuring a first parameter for each one of
25   the plurality of different particles. One of the
particles is identified based upon the first
parameter measured for the one particle. A second
parameter, different from the first parameter
associated with the one particle, is then measured
30   for the one particle identified. The other particles
in the field of view are identified bas d upon the
second paramet r associated with the one particle
that has been identified.

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Brief Description Of The Drawings

Fig. 1 is a schematic diagram of an image of a view with a plurality of different types of particles shown.

5   Detailed Description Of The Drawings

Referring to Fig. 1, there is shown an image 10 of a plurality of particles in a field of view. Although the method of the present invention will be described with respect to biological particles in a

10   field of view, it can be appreciated that the method of the present invention can be practiced with any type of particles or with any type of members in a group of members.

As shown in Fig. 1, a plurality of different

15   types of particles (A...H) are shown in the image 10. Thus, there are three particles of type A, three particles of type B, two particles of type C ... and two particles of type H. However, when an image 10 of a plurality of particles is presented, the type of

20   particles is not a priori known. Further, statistical variations in certain parameters of a type of particle may exist. For example, a type A particle may be "larger" in size than the particles of type B and type C. However, not all particles of

25   Type A may be of the same size. The method of the present invention distinguishes the type of particles and identifies them in the image 10 in the field of view.

As used herein, the term "identify" means to

30   determine the label or identifier which is attached to a particular particle or group of particles. Further the term also includes the characteristics, e.g. statistical variations, of the identified group

of particles.   The term "distinguish" or
"differentiate" means to separate a group of
particles into two or more subpopulation groups based
upon one or more parameters. The separation,
however, does not necessarily involve identification
of the subpopulation groups, i.e. determination of
the label for each of the subpopulation groups.

In the method of the present invention, a first
parameter is measured for each one of the plurality
of particles in the image 10. The first parameter
can be a parameter such as color.

Based upon the color intensity of the various
particles, one of the type of particles, such as
particle A, is identified. Thereafter, a second
parameter, different from the first parameter, is
measured for the particles which have been
identified; namely, particles A. The second
parameter can be a morphological parameter; such as
size, which is different from spectral parameter such
as color. Thus, the particles A identified are then
measured for size.

Finally, the other particles in the field of
view 10 are identified, based upon the second
parameter. The second parameter is measured for each
of the other particles. The measurement of the
second parameter (for each of the other particles,
other than particles identified as being of type A)
is compared to the measurement of the parameter
determined for the particles identified as being of
type A. Based upon this comparison, the other
particles are then identified. Thus, for example,
the morphological characteristic of size for all the
other particles is then compared to the size of
particle type A, and is identified based upon this

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comparison. If the size of a particle exceeds that
of the size for particles of type A, then the
particle is identified as being of a certain type,
such as type B.

    It should be noted while the method has been
described with regard to the first parameter, which
is measured as being a spectral parameter and the
second parameter as being a morphological parameter,
the method of the present invention can also be used
where the first parameter is a morphological
parameter and wherein the second parameter is a
spectral parameter.

    The method of the present invention will now be
described with specific reference to the following
examples:

EXAMPLE 1

    In a biological sample containing lymphocytes,
monocytes and neutrophils, it is desired to
separately identify all of these different types of
particles. However, the difference between monocytes
and neutrophils cannot be distinguished based upon
color because they differ from sample to sample.
Thus, in the method of the present invention, the
size of all the different particles is first
measured. Lymphocytes are then distinguished from
monocytes and neutrophils by the size of the
Lymphocyte particles. If the size of the particles
is less than approximately 50 μm^2 (200 .5x.5μm pixel),
for example, then the particles are deemed to be
lymphocytes, whereas all other particles contain both
monocytes (if any) and neutrophils (if any).

    Thereafter, the color of the lymphocytes so
identified is measured. The average color of all the
lymphocytes identified is measured. The color

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measured for the identified lymphocytes is then used
as a threshold to differentiate monocytes from
neutrophils. If the color of a particular particle
is greater than the average color of the lymphocytes,
5   then the particle is a neutrophil. If the color of
the particle under examination is less than the
average color of the identified lymphocytes, then the
particle under examination is a monocyte. In this
manner, monocytes and neutrophils can be
10   distinguished from one another and identified based
upon the average color of lymphocytes in the same
sample in which the monocytes and the neutrophils
exist.

EXAMPLE 2

15   A plurality of different types of particles
including basophils are shown in an image in a field
of view. The basophils are identified by color from
all the other types of particles by conventional
means or by the use of zeroth green quantile for B021
20   stained cells. For a description of quantile
measurement, see co-pending U.S. application 357,324,
filed on May 25, 1989 and assigned to the same
assignee as the present invention. That co-pending
application is incorporated herein by reference.
25   Thereafter, the area of the identified basophils is
measured in pixels and the average area of all
basophils thus identified is computed. From the
average area of the measured basophils, the pixel
size can be determined, assuming a nominal basophil
30   area of about 42 μm^2. From the pixel size determined,
the other particles (such as neutrophils, eosinophils
and monocytes) can be distinguished based upon this
pixel size by computing all particle areas and
assigning all particles with areas greater than $50 \mu m^2$

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to the neutrophil, eosinophil and monocyte category.
Alternatively, this decision may be based on the area
of a particle relative to the basophil average.

EXAMPLE 3

5   A plurality of different types of particles
including known cancer cells and cells that are
potentially cancerous are in the imaged field of
view. The plurality of different types of particles
are subjected to reaction with an antibody which has
10   a color tag attached thereto. The antibody would
attach itself to the known cancer cells. Those known
cancer cells would have a particular color, depending
on the color tag attached to the antibody. The
colored cells are then located and the ploidy of the
15   known cancer cells are measured. The ploidy of the
subpopulation of known cancer cells would have a
statistical distribution. Based upon this measured
ploidy, the potentially cancerous cells are
differentiated from the healthy cells. That is, the
20   ploidy of the potentially cancerous cells and of the
healthy cells are also measured. If the ploidy of a
measured unknown cell is approximately on the same
order (i.e. within the statistical distribution) as
the ploidy of the known cancer cells, then that cell
25   is potentially cancerous. On the other hand, if the
ploidy of a measured unknown cell is far less than
the ploidy (i.e. outside of the statistical
distribution) of the measured known cancer cells,
then the unknown cell is a healthy cell. In this
30   manner, healthy cells can be distinguished from
potentially cancerous cells.

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EXAMPLE 4

A gene probe for human papilloma virus (HPV) is
hybridized to a plurality of different cells. The
gene probe has an enzyme label attached thereto.
5   Upon introduction of substrate material, the
substrate is converted by the enzyme and produces a
local colored deposit within the cell. The ploidy of
the HPV population (identified by the presence of the
colored deposits) may be measured and compared to the
10 ploidy of the non-HPV population (identified by the
absence of colored deposits) to determine the degree
to which the HPV population has changed from normal.

The theory of operation of the method of the
present invention is as follows. The invention is of
15 a method which determines a dynamically changeable
normalization threshold from which other particles
can be differentiated. Unlike the prior art where
the threshold is fixed or is a priori known, the
method of the present invention recognizes that the
20 threshold for each sample would differ depending on
factors such as age and color stain which vary from
sample to sample. Thus, the method of the present
invention overcomes the problem of experimental
variability; therefore, the same method can be used
25 to differentiate cells in different experiments where
the experiments of the different samples are subject
to different variables.

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WHAT IS CLAIMED IS:

1.   A method of identifying a plurality of
different types of particles in a field of view,
comprising the steps of:

5   a)   measuring a first parameter for each
of said plurality of different particles;
               b)   identifying one of said type of
particle based upon the first parameter
measured;

10   c)   measuring a second parameter different
from said first parameter, for each of said
plurality of different particles; and
               d)   identifying other particles in said
field of view based upon said second parameter

15   associated with said one type of particle
identified.

2.   The method of Claim 1, wherein said first
parameter is a morphological parameter and wherein
said second parameter is a spectral parameter.

20   3.   The method of Claim 1, wherein said first
parameter is a spectral parameter and wherein said
second parameter is a morphological parameter.

4.   The method of Claim 1, wherein said
identifying step of (d) further comprising:

25   measuring the second parameter of other
particles in said field of view; and
               using the measured second parameter
associated with said one particle to identify
other particles in said field of view.

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        5.   A method of differentiating a first type of
cell in a plurality of fields of view where the first
type of cell in different fields of view differ in a
first detectable parameter, wherein the same method
5   is used to differentiate the first type of cell from
other cells in each field of view, said method
comprising the steps of:
                a)   selecting a field of view;
                b)   measuring a second parameter of each
10   of said cells in said selected field of view;
                c)   identifying one of said cells, other
than the first cell, based upon said second
parameter measured;
                d)   measuring said first detectable
15   parameter, different from said second parameter,
associated with said identified one cell; and
                e)   differentiating said first cell from
other cells based upon the first parameter
measured for said identified one cell.

20   6.   The method of Claim 5, wherein said first
parameter is a morphological parameter, and wherein
said second parameter is a spectral parameter.

        7.   The method of Claim 5, wherein said first
parameter is a spectral parameter, and wherein said
25   second parameter is a morphological parameter.

        8.   The method of Claim 5, wherein said
differentiating step of (e) further comprising:
                measuring the first parameter of the other
cells in the field of view;
30   comparing the first parameter of each cell
measured to the first parameter of said

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identified one cell and differentiating said
first cell from said other cells based upon said
comparing.

9.   A method of identifying a first plurality
of members (hereinafter: "a first sub-population")
from a group of members, comprising the steps of:
        measuring a first characteristic of each
members of said group;
        identifying a second plurality of members
(hereinafter: "a second sub-population") from
said group based upon said first characteristic
measured;
        measuring a second characteristic,
different from said first characteristic, of
each member of said group; and
        identifying the members of said first sub-
population based upon the measured second
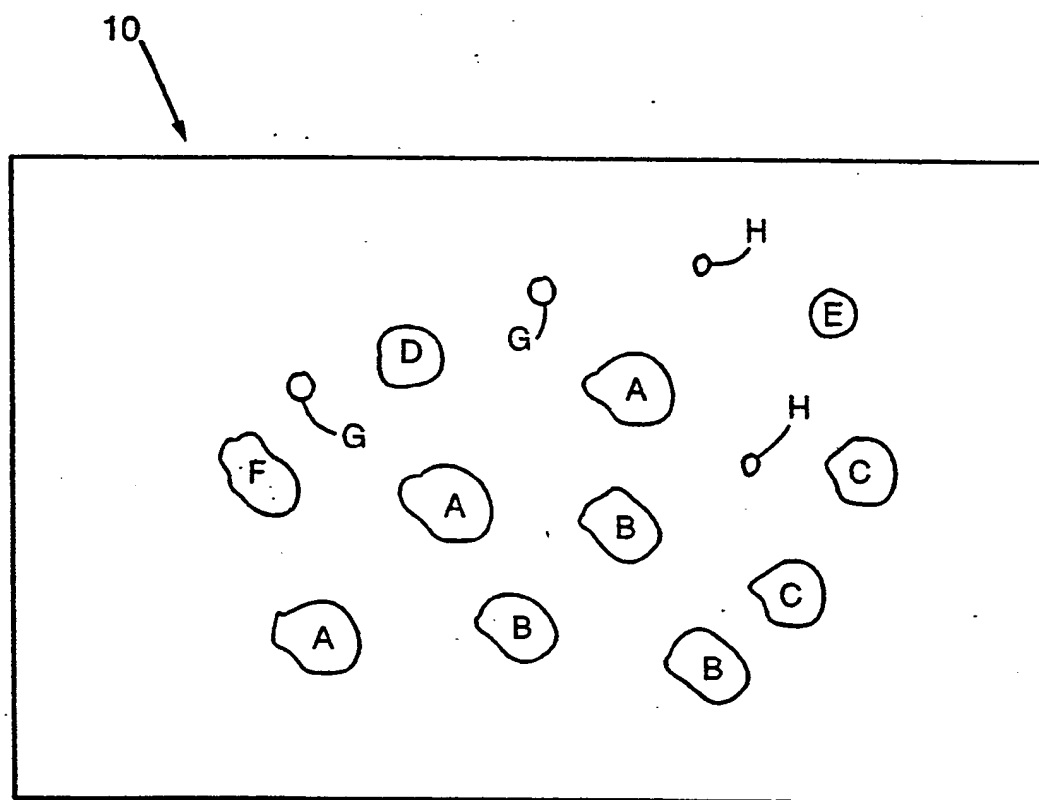characteristic of each member of said second
sub-population.

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FIG. 1

INTERNATIONAL SEARCH REPORT

International Application No   PCT/US90/04446

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 3

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5) : G06K 9/00
U.S. Cl : 382/6

II. FIELDS SEARCHED

Minimum Documentation Searched 4	
Classification System	Classification Symbols
U.S.	356/39; 382/1,6,17,25

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched 5

III. DOCUMENTS CONSIDERED TO BE RELEVANT 14

Category *	Citation of Document, 16 with indication, where appropriate, of the relevant passages 17	Relevant to Claim No. 18
X	US,A 4,338,024 (BOLZ ET AL) 06 July 1982. See column 1, 65-69; Column 2, lines 1-11; column 4, lines 67-69.	1-9
A	US,A 4,665,553 (GERSHMAN ET AL) 12 May 1987 See column 3, lines 35-44.	1-9
A	US,A 4,661,913 (WU ET AL) 28 April 1987 See column 4, lines 34-41.	1-9
A	US,A 4,097,845 (BACUS) 27 June 1978 See column 1, lines 58-69; column 4, lines 1-4.	1-9

* Special categories of cited documents: 15

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"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search 2	Date of Mailing of this International Search Report 2
10 SEPTEMBER 1990	09 JAN 1991
International Searching Authority 1	Signature of Authorized Officer NGUYEN NGOC-HO INTERNATIONAL DIVISION
ISA/US	STEVEN P. FALLON

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